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Age structure of a population of *Barbarophryne brongersmai* (Hoogmoed 1972) (Anura, Bufonidae) inhabiting an arid environment in the Central Jbilets (West-Morocco)

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Abstract. We estimated age structure and some life-history traits in a population of *Barbarophryne brongersmai* at the northern border of the distribution area of this species by using skeletochronology to establish individual age and age at sexual maturity. Maturity was reached at 2-4 years in males and 3-5 years in females, which was late relatively to small body size. Longevity was estimated to be 12 and 8 years for males and females, respectively, whereas mean body size is 45.6 mm in both sexes. However, this estimate suffers from small sample size in females. We did not detect a positive relationship between age and size, suggesting that adult individuals cease growing after maturity. Conversely, body size varied within an age class, suggesting great variability of growth rate among individuals during the juvenile stage. Collectively, these traits characterize a life history strategy that could be an evolutionary response to habitat unpredictability in arid regions.

Keywords. Endemic species, skeletochronology, life history traits, arid environments, Morocco

In arid zones, the reproduction of the amphibians is exposed to high environmental stochasticity because both the amount of rainfall and the hydroperiod of the breeding sites are usually variable and unpredictable. We assume that increased probability of reproduction failure due to pond drying is compensated by higher longevity as suggested by life history theory (Roff, 1992) and already shown in amphibians (Joly and Morand, 1994; Church et al., 2007; Cayuela et al., 2014). In the perspective of increasing the data set about this issue, we investigated the age structure of a small-bodied toad, *Barbarophryne brongersmai*, at the northern edge of the Sahara desert to compare it with those established in other Bufonidae breeding in less unpredictable environments.

Described and separated from *Bufotes viridis* by Hoogmoed (1972), *Barbarophryne brongersmai* is a small-bodied toad endemic to south-western Morocco (in the present paper, we consider recent taxonomic revisions of the *Bufo* genus by Pyron and Wiens, 2011, and Beukema et al., 2013). The studied population inhabits an arid grassland with sparsely scattered jujube trees (*Ziziphus lotus*), located 25 km north of Marrakech in the central Jbilets (31°37'N, 8°02'W), a range of low hills near the northern border of the distribution area of the species (Guillon et al., 2004). In this region, annual precipitation rarely reaches 300 mm (Le Houérou, 2001) and rare rain events of short duration and high intensity provoke sudden water discharges in the wadis (temporary rivers), usually occurring from the end of February to mid-April. The studied area is heavily grazed by large herds of sheep and goats in permanent movement that keep the grass very short, even during the rainy season. Our popula-

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tion breeds at a temporary pond (15 m in diameter and 80 cm depth) that appears in the bed of a temporary river (Defla wadi), the hydroperiod of which did not exceed 18 days from 2009 to 2012 because of strong evaporation and intensive use for livestock watering.

Because the toads come to the pond immediately after it fills with water (Guillon et al., 2004; Fattah, 2008; Slimani et al., 2012), sampling occurred from February to April, at night (from 20 to 00h GMT) using hand nets. We measured the toads from snout to cloaca by means of a calliper and weighed them using a field balance (accuracy 0.1g). We determined gender by the presence of nuptial callosities and calling in males. After measurements, we collected the third toe of the hind leg and we immediately released the toad at the place of its capture. We sectioned phalanges in order to quantify numbers of lines of arrested growth (LAGs), following the method by Castanet and Smirina (1990) based on the detection of line of arrested growth generated by the cold and/or dry season (each LAG is interpreted as 1 year of age). The studies by Barbault et al. (1979) on "*Bufo"pentoni*, Esteban et al. (1999) on *Pelophylax saharicus*, and Doglio et al. (2008) on *B. brongersmai* have confirmed that long bones reliably record yearly growth arrest in arid regions bordering Sahara desert. However, this method is suspected to regularly underestimate age because of bone remodelling and rapprochement of peripheral lines, especially in the oldest individuals (Wagner et al., 2011). We made sections in the medium part (diaphysis) of the third phalanx because the two distal phalanxes are known to exhibit strong medullary resorption (Francillon-Vieillot, 1987; Fretey and Le Garff, 1992). All the sections where age estimation was doubtful (because of medullary resorption or other cause) were discarded from the results. Three observers (A.F., O.G. and P.J.) independently counted the LAGs of each individual and re-examined the specimen and discussed the results when their estimations diverged. When the result remained uncertain, the specimen was not taken into account. Age at maturity was estimated by the sudden and permanent rapprochement of the LAGs that reflects the slowing of growth rate after sexual maturity is reached.

Data normality was first assessed (Kolmogorov-Smirnov test) before testing for difference between means with Mann-Whitney U test, while correlation between age and body size was assessed by Spearman test. Means are reported with standard deviation and tests were considered significant at $\alpha = 0.05$.

During the 4-years survey, we sampled 80 individuals (71 males and 9 females, sex ratio = 0.89), but we succeeded in establishing age in only 37 males and 6 females. The bone sections show clear lines of arrested growth (LAGs) that are almost circular and continuous in most individuals (68.6%, Fig. 1). We considered the perimeter of the bone as corresponding to the last winter since the toads were sampled in February, March and

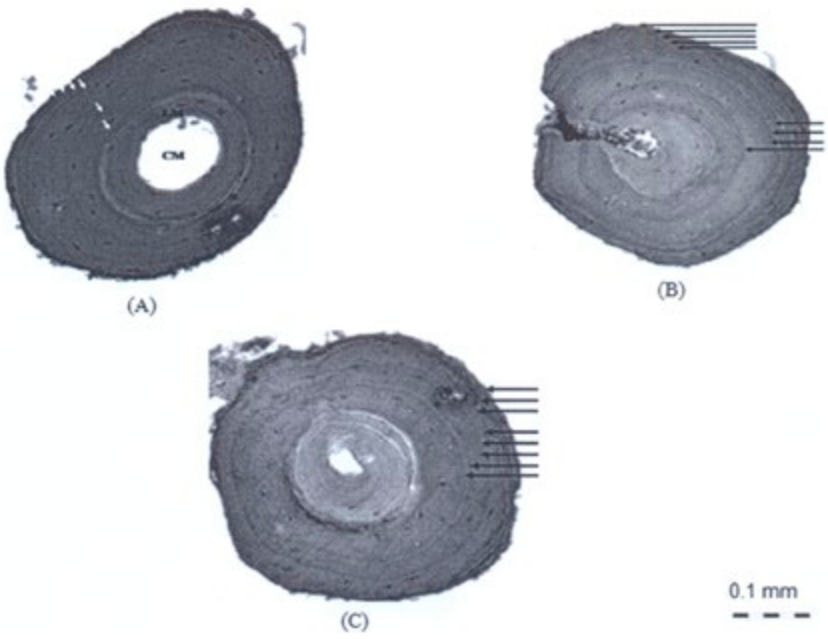

Fig. 1. Transverse sections within the diaphysal region of phalanx bones in the studied population. (A) Male with 6 visible LAGs (CM: medullary cavity; LM: line of metamorphosis). (B) Male with 9 visible LAGs. (C) Female with 8 visible LAGs.

April. We clearly detected the decrease in the distance that separates the LAGs suggesting that sexual maturity is reached at 2-4 years in males, and 3-5 years in females (Fig. 1B, C), although their low number prevents any firm conclusion. At the breeding site, we sampled some males younger than 4 years, although 4-year individuals were the most frequently observed (Fig. 2). Highest estimated age is 12 years in males and 8 years in females.

Body size varied from 35.5 to 52.2 mm in males (45.4 ± 2.9 mm, n = 37) and from 40.0 to 50.5 mm in females (45.6 ± 4.2 mm, n = 6), with a mass of 6.5 ± 1.2 g in males and 7.4 ± 1.9 g in females and the observed pattern of sexual dimorphism in body size was not significant (Mann–Whitney *U*-test: $U = 110.5$, Z=0.017, P = 0.10). Because we did not detect a significant relationship between age and body size in males ($r = -0.073$, P = 0.667, n = 37), or in females ($r = 0.529$, P = 0.279, n = 6) (Fig. 3), the oldest individuals were not always the largest ones.

Our study provides data about population structure that brings new light on the adaptive strategy of this species, despite the low confidence of our conclusions about growth and longevity in females, because of the low numbers of sampled individuals. This apparent bias in sex ratio could be due to (i) higher detectability of males because of conspicuous breeding behaviour, (ii) longer stay at breeding site, (iii) visiting the breeding location too early after rainfalls for the females to be present, and (iv) the possibility that the reproductive cycle of females is biennial when that of males is annual. Sexual maturity is reached after about 3-4 years in most males and 5 years in females. However age structure in males is skewed since we would expect the 2 and 3 years-old toads to be more numerous than those aged 4 as a result of mortality. A first hypothesis for explaining this gap in young males is that not all the males reach sexual maturity before reaching 4 years, whereas a second one is that some males reach sexual maturity when two years old. We do not have enough evidence to conclusively reject either of these hypotheses.

The one-year difference in age at maturity between males and females is commonly observed in the anurans, and is usually attributed to the higher investment of females in gametes (Miaud et al., 1999; Bastien and Leclair, 1992; Kyriakopoulou-Sklavounou and Grumiro, 2002; Eaton et al., 2005). However, males and females can reach sexual maturity at the same age in some toads and other anurans (Lykens and Forester, 1987; Friedl and Klump, 1997; Kyriakopoulou-Sklavounou et al., 2008), probably when resources are abundant. The late age at maturity in our population suggests a low growth rate in juveniles, strongly constrained by the shortness of the activity period and perhaps the scarcity of food. As a consequence, the difference in age at maturity between genders is not surprising in such an environment. However, the low number of female sampled prevents any firm conclusion about this supposed lack of difference between genders.

The absence of a relationship between age and body size is surprising because anurans often exhibit continuous growth, particularly females because of the strong relationship between fecundity and body size (Joly, 1991; Prado and Haddad, 2005; Lengagne et al., 2007). Increasing fecundity at the expense of reducing somatic growth is theoretically possible, leading thus females keeping a

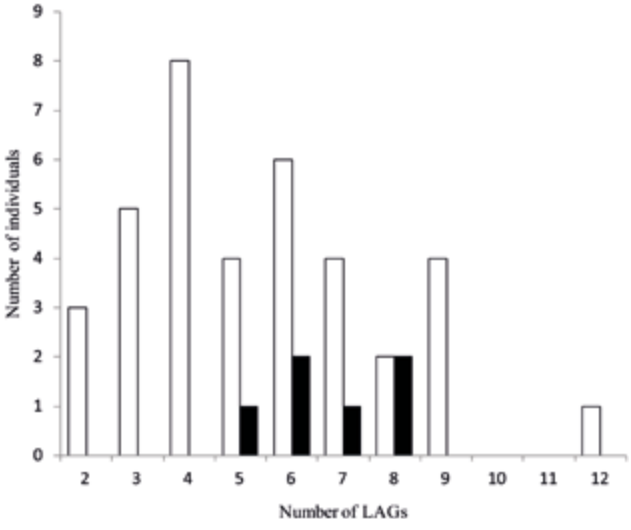

Fig. 2. Frequency of *Barbarophryne brongersmai* at Defla wadi in 2009-2012 according to estimated age in the studied population. Filled bars: females; open bars: males.

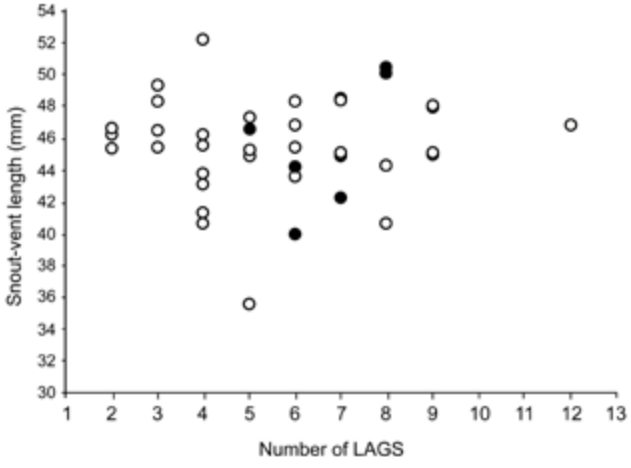

Fig. 3. Relationship between estimated age (X-axis in year) and body size (Y-axis; snout-vent length in mm) in the studied population. Dark dots: females; open dots: males.

Table 1. Comparison of longevity and age at maturity in several Bufonids.

Species	Longevity (year)	Age at maturity (year)	Body size (average mass of the species/ average mass of *Barbarophryne brongersmai*)	Remarks	Data source
Bufo bufo	8	2-4	3 to 4	Higher at elevation >1800m	Hemelaar, 1988; Frazer, 1966; Fretey, 1995; Gittins et al., 1980; Gittins et al., 1982
Epidalea calamita	9	2-3	2	Higher at elevation > 2200 m	Stevens et al., 2003; Leskovar et al., 2006; Oromi et al., 2012
Bufotes viridis	9	2-5	2	-	Sinsch et al., 2004
Anaxyrus hemiophrys	9	1-2	-	-	Eaton et al., 2005

constant body size while increasing reproductive investment when ageing (Castellano et al., 2004). However, such a theoretical option of resource allocation dynamics is not common in the amphibians that usually increase fecundity along life through indeterminate growth. Furthermore, the absence of sexual dimorphism in body size also suggests a selection for low annual fecundity that could be compensated by a longer lifespan. In males, the observed longevity exceeds by 4 years that estimated in Jbel Saghro (Anti Atlas, Morocco) by Doglio et al. (2008). This greater longevity in the Central Jbilets could be due to greater sampling effort in our population. By visually extrapolating the decreased number of individuals as age increases, we predict that life expectancy could reach 13-14 years in the studied population. Longevity (potential lifetime under optimal life conditions) should be longer first because skeletochronology usually underestimates current age due to bone remodelling and rapprochement of peripheral lines (Wagner et al., 2011) and second because natural populations are exposed to external causes of mortality (predation, disease, drought, decreased food availability) that reduce life expectancy. In other anurans, longevity estimated in the field is around threefold lower than that measured under optimal rearing conditions. In *Bombina variegata* for example, longevity reaches 29 years in artificially reared individuals when it does not exceed 12-13 years in the field (Plytycz and Bigaj, 1993; unpubl. res.). We thus suspect *B. brongersmai* to have a potential longevity much higher than expected on the basis of its body size. This hypothesis is supported by the fact that most toad species with body size larger than that of *B. brongersmai* exhibit shorter longevity and smaller age at maturity even in species with higher body size (Table 1). These comparisons suggest that small-bodied *B. brongersmai* is characterized by slow growth during the juvenile stage.

The interplay of high longevity, slow growth during the juvenile stage, absence of continued growth at the adult stage, and small body size constitutes a specific demographic strategy. We assume that this life history style constitutes a response to the unpredictability of breeding habitats and the shortness of activity period by increasing the number of lifetime breeding occasions (Seger and Brockmann, 1987; Roff, 1992). The test of this hypothesis needs to establish age structure in other populations, including large numbers of females, and to investigate physiological mechanisms that support the great longevity of this species.

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